

In the Specification

Please substitute the following title of the invention on page 1:

COMPLETE BIOSYNTHETIC GENE SET FOR SYNTHESIS OF POLYKETIDE  
ANTIBIOTICS, INCLUDING THE ALBICIDIN FAMILY, RESISTANCE GENES, AND  
USES THEREOF

BIOSYNTHETIC GENES AND HOST CELLS FOR THE SYNTHESIS  
OF POLYKETIDE ANTIBIOTICS AND METHOD OF USE

Please substitute the following first paragraph on page 1:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the U.S. national stage application of International Patent Application No. PCT/US2003/33142, filed October 17, 2003, which claims the benefit of U.S. Provisional patent application with Serial No. 60/419,463, filed October 18, 2002 the disclosure disclosures of which is are hereby incorporated by reference in its their entirety, including all nucleic acid sequences, amino acid sequences, chemical formulae, tables and figures.

Please insert the following paragraph on page 1, before paragraph [0001]:

The Sequence Listing for this application is labeled "seq-list-replace.txt" which was created on June 26, 2008 and is 323 KB. The entire contents of the sequence listing is incorporated herein by reference in its entirety.

Please substitute paragraphs [0015], [0016], [0017] and [0018] beginning on page 4 as follows:

**Figure 5** is an illustration of the alignment of the primary sequences between the conserved motifs A4 and A5 of Alb NPRSs and PKS-4 in *Xanthomonas albilineans* with the corresponding sequences of GrsA (Phe) accession number: P14687 (SEQ ID NO: 132) and Blm NRPS-2 ( $\beta$ -Ala) accession number AF210249 (SEQ ID NO: 133); AlbI NRPS-1 (SEQ ID NO: 134); AlbI NRPS-3 (SEQ ID NO: 135); AlbIX NRPS-6 (SEQ ID NO: 136); AlbIX NRPS-7 (SEQ ID NO: 137); AlbIV

NRPS-5 (SEQ ID NO: 138); AlbVII PKS-4 (SEQ ID NO: 139); AlbI NRPS-2 (SEQ ID NO: 140).

**Figure 6** shows Rho-independent transcription terminators identified in the intergenic regions of XALB1 and XALB3 clusters (SEQ ID NO: 141, XALB1 Strand + (29 bp downstream from the TGA stop codon of *albXVII*); SEQ ID NO: 142, XALB1 Strand + (400 bp downstream from the TAA stop codon of *albII*); SEQ ID NOs: 143, 144 and 145, XALB1 Strand – (62 bp, 170 bp and 560 bp downstream from the TAG stop codon of *albXVI*); SEQ ID NOs: 146 and 147, XLAB3 Strand+).

**Figure 7A** shows sequences identified as a putative bidirectional promoter between *albX* and *albXVII* in XALB1 for transcriptional control of operons 3 and 4 (SEQ ID NOs: 148 and 149).

**Figure 7B** shows sequences identified as a putative unidirectional promoter upstream from *albXIX* for transcriptional control of operon 5 if *albXVIII* is not expressed (SEQ ID NOs: 152 and 153).

Please substitute paragraphs [0022] and [0023] beginning on page 5 as follows:

**Figure 10A** is an alignment of the conserved motifs in AT domains from RifA-1 (SEQ ID NO: 156), -2 (SEQ ID NO: 157), -3 (SEQ ID NO: 158), RifB-1 (SEQ ID NO: 159), RifE-1 (SEQ ID NO: 160) (Rifamycin PKSs, August *et al.*, 1998) and BlmVIII (Bleomycin PKS; Du *et al.*, 2000) (SEQ ID NO: 161).

**Figure 10B** is a comparison of AlbXIII (SEQ ID NO: 162), FenF (a malonyl-CoA transacylase located upstream from *mycA*, Duitman *et al.*, 1999) (SEQ ID NO: 163) and LipA (a lipase; Valdez *et al.*, 1999) (SEQ ID NO: 164).

Please substitute paragraph [00120] beginning on page 35 as follows:

*albII* potentially encodes a protein of 343 aa (AlbII) with a predicted size of 37.7 kDa. *albII* is 100% identical to the *xabC* cistron, previously described as encoding an *O*-methyltransferase downstream *xabB* (Huang *et al.*, 2000a). This conclusion is based on the similarity of XabC with a family of methyltransferases that utilize S-adenosyl-L-methionine (SAM) as a co-substrate for *O*-methylation including TemO protein from *Streptomyces glaucescens* (Huang *et al.*, 2000a). AlbII contains three highly conserved motifs of SAM-dependent methyltransferases, including the motif I

involved in SAM binding (Figure 3). In the Figure, identical or similar amino acids (A=G; D=E; I=L=V) are shown in bold. Numbers indicate the position of the amino acid from the N-terminus of the protein. Abbreviations used in the Figure are: Sgl-TcmO (SEQ ID NOs: 55, 56 and 57) and Sgl-TcmN (SEQ ID NOs: 58, 59 and 60), multifunctional cyclase-hydratase-3-O-Mtase and tetracenomycin polyketide synthesis 8-O-Mtase of *Streptomyces glaucescens*, respectively (accession number: M80674); Smy-MdmC, midecamycin-O-Mtase of *Streptomyces mycarofaciens* (accession number: M93958) (SEQ ID NOs: 61, 62 and 63); Mxa-SafC, Saframycin O-Mtase of *Mycrococcus xanthus* (accession number: U24657) (SEQ ID NOs: 64, 65 and 66); Ser-EryG, erythromycin biosynthesis O-Mtase of *Saccharopolyspora erythraea* (accession number: S18533) (SEQ ID NOs: 67, 68 and 69); Spe-DauK, carminomycin 4-O-Mtase of *Streptomyces peucetius* (accession number: L13453) (SEQ ID NOs: 70, 71 and 72); Sal-DmpM, O-demethylpuromycin-O-Mtase of *Streptomyces alboniger* (accession number: M74560) (SEQ ID NOs: 73, 74 and 75); Shy-RapM, rapamycin O-Mtase of *Streptomyces hygroscopicus* (accession number: X86780) (SEQ ID NOs: 76, 77 and 78); Sav-AveD, avermectin B 5-O-Mtase of *Streptomyces avermitilis* (accession number: G5921167) (SEQ ID NOs: 79, 80 and 81); Sar-Cmet, mithramycin C-methyltransferase of *Streptomyces argillaceus* (accession number: AF077869) (SEQ ID NOs: 82, 83 and 84); AlbII, putative albicidin biosynthesis C-Methyltransferase of *Xanthomonas albilineans* (SEQ ID No. 27); identical to XabC, accession number: AF239749) (SEQ ID NOs: 85, 86 and 87).

Please substitute paragraph [00122] beginning on page 35 as follows:

*albVI* potentially encodes a protein of 286 aa (AlbVI) with a predicted size of 32.1 kDa similar to several hypothetical protein from *Mycobacterium tuberculosis* (Genbank accessions No. AAK46042, AAK48238, AAK44517, AAK46218) and from *S. coelicolor* (Genbank accession No. CAC03631). AlbVI is also similar to the tetracenomycine C synthesis protein (TcmP) of *Pasteurella multocida* (Table 4). Four highly conserved motifs in TcmP and other O-methyltransferases are also present in AlbVI (Figure 4), suggesting that AlbVI is an O-methyltransferase. In the Figure, identical or similar aa (A=G; D=E; I=L=V; K=R) are shown in bold. Numbers indicate the position of aa from the N-terminus of the protein. Abbreviations used in the Figure are: Sgl-tcmP, tetracenomycin C synthesis protein of *Streptomyces glaucescens* (accession number: C47127) (SEQ ID NOs: 88, 89,

90 and 91); Sme-PKS, putative polyketide synthase of *Sinorhizobium meliloti* (accession number: AAK65734) (SEQ ID NOs: 92, 93, 94 and 95); Pmu-tcmP: tetracenomycin C synthesis protein of *Pasteurella multocida* (accession number: AAK03406) (SEQ ID NOs: 96, 97, 98 and 99); Mtu-Omt: putative *O*-methyltransferase of *Mycobacterium tuberculosis* (accession number: AAK45444) (SEQ ID NOs: 100, 101, 102 and 103); Mlo-Hp: hypothetical protein containing similarity to *O*-methyltransferase of *Mesorhizobium loti* (accession number: BAB50127) (SEQ ID NOs: 104, 105, 106 and 107); ~~Mtu-Hp1~~ Mtu-Hp: hypothetical protein of *Mycobacterium tuberculosis* (accession number: AAK46042) (SEQ ID NOs: 108, 109, 110 and 111); Mtu-Hp2: hypothetical protein of *Mycobacterium tuberculosis* (accession number: AAK48238) (SEQ ID NOs: 112, 113, 114 and 115); Mtu-Hp3: hypothetical protein of *Mycobacterium tuberculosis* (accession number: AAK44517) (SEQ ID NOs: 116, 117, 118 and 119); Mtu-Hp4: hypothetical protein of *Mycobacterium tuberculosis* (accession number: AAK46218) (SEQ ID NOs: 120, 121, 122 and 123); Sco-Hp: hypothetical protein of *Streptomyces coelicolor* (accession number: CAC03631) (SEQ ID NOs: 124, 125, 126 and 127); AlbVI, putative albicidin biosynthesis *O*-Methyltransferase of *Xanthomonas albilineans* (this study) (SEQ ID NOs: 128, 129, 130 and 131).

Please replace pages 1-93 (Sequence Listing) submitted on April 25, 2005 in the subject application with new pages 1-180 submitted herewith.